

K951014

ISOLAB INC.

510(K)

T4 TEST KIT
EXHIBIT C

OCT 18 1996

Summary of Safety and Effectiveness

Isolab's T4 Test Kit is intended for use in determining the total thyroxine (T4) in blood specimens dried on filter paper.

The Isolab's T4 Test Kit is a sequential, competitive enzyme immunoassay with fluorometric detection in which T4 is eluted from dried blood disks and simultaneously forms a complex with the biotin-labeled monoclonal antibody which binds to the streptavidin coated solid phase. After sufficient incubation with biotinylated antibody, a conjugate of T4 with horseradish peroxidase is added to the wells and incubated with the reaction mixture. This conjugate competes with the analyte for the free binding sites on the bound monoclonal antibody. Excess of unbound reagent is washed away after a sequential incubation with biotinylated antibody and conjugate, and the enzymatic reaction with a fluorogenic substrate 3-p-hydroxyphenylpropionic acid (HPPA) [9] takes place. The reaction is stopped by addition of glycine buffer and the fluorescence in each well is measured with Fluoroscan II Neonate at 405 nm (excitation being 320 nm).

Isolab has two kit sizes available for the T4 Test Kit. Test Kit NT-1000 contains enough reagents and Microstrips to run 800 patient samples. Additional wells are available for Calibrators and controls. Test Kit NT-4000 contains enough reagents and coated Microstrips to run 4000 patient samples. Additional wells are available for Calibrators and Controls.

Code	Code	Description
NT-1000	NT-4000	Item
10 plates	50 plates	Streptavidin coated Microstrips®
2 x 90 ml	10 x 90 ml	Biotinylated Antibody (Bottle 1)
2 X 30 ml	10 X 30 ml	T4-HRP Conjugate (Bottle 2)
210 ml	5 X 210 ml	HPPA Substrate (Bottle 3)
45 ml	5 X 45 ml	H ₂ O ₂ Solution (Bottle 4)
200 ml	2 X 500 ml	Stopping Solution (Bottle 5)
220 ml	2 X 500 ml	Wash Solution (Bottle 6)
1 card	5 cards	T4 Calibrators
10	50	Microstrip Covers (Plastic sheets to cover the Microstrips® during incubation.)

The T4 values given for the Controls, Calibrators and thus patient results are given in µg/dL. This unit of measure reflects the estimated amount that would be found in serum. These values should be considered accurate for the purposes of screening. Due to differences in hematocrit values, these values should not be assumed as accurate as those from serum-based assays. The discrimination between normal and presumptive positives for CH is based on a predetermined fixed cut-off value generally regarded to be based on the lower 10%. The cut-off value that represents the lower 10% for this assay is 8.5 µg/dl. Any result at this value or below should be confirmed by another diagnostic test or procedure to confirm that the patient is positive for congenital hypothyroidism.

Linearity

Results of the linearity study show that the assay is linear from concentration of 0 to 25 µg/dL of T4. Concentrations of T4 in the amounts of 6.25, 12.5, and 25 µg/dL were added to a whole blood sample and spotted onto filter Schleicher & Schuell No. 903 paper. Each spiked sample along with the unspiked sample was run five times.

Recovery

The recovery was determined from the same data as shown under Reproducibility. The recovery can be calculated by the following formula:

$$\frac{\text{mean}}{\text{amount added}} \times 100 = \% \text{ recovery}$$

$$\frac{5.5}{6.25} \times 100 = 88\%$$

$$\frac{12.1}{12.5} \times 100 = 97\%$$

The recovery is shown at the low T4 levels to be around 88% and at normal levels around 97%.

Sensitivity

The detection limit for the T4 assay 1.0 µg/dL.

Reproducibility

Two sample pools were run in duplicate twice a day for 20 days. The summary of the results are shown:

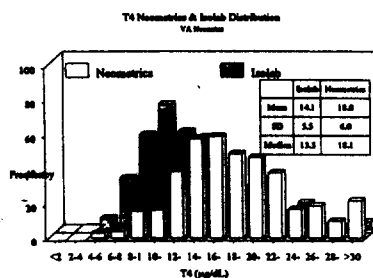
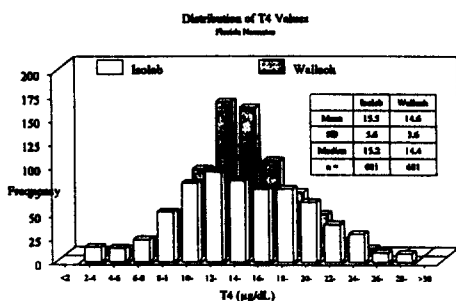
	amount added	mean	total SD	total CV	within-run SD	within-run CV
T4 depleted neonate	6.25 µg/dL	5.5 µg/dL	0.57	10.4%	0.43	7.8%
normal neonate	12.5 µg/dL	12.1 µg/dL	1.89	15.6%	1.52	12.6%

Comparison of Methods

Isolab's T4 Test Kit was compared to Neometric's Accuscreen T4 and Wallac Oy's Delphia Neonatal T4. The values for the Neometrics and Wallac Oy assays were performed at two state laboratory facilities. the lower 10% of the values for each of the assays are shown.

Test	State 1 Cutoff (µg/dL)	State 2 Cutoff (µg/dL)
Isolab	8.5	8.1
Neometrics	-	11.7
Wallac Oy	10.2	-

The distribution of values as well as the mean values, standard deviations and median values for the Isolab methods and the two comparative test kit methods are shown on the next page.



Interference testing was designed and executed using NCCLS document EP-7 as guidance [14]. The following substances were added to a control sample and tested as possible interferences to the T4 Test Kit. The control sample was assayed seven times and each of the whole blood samples spiked with the following substances were also assayed seven times. The two thyroxines (D-T4 and DL-T4) added about 100% to the calculated values as expected. Two other thyroxine analogs (DL-T₀ and TA₃) also produced a slight interference. The drug, 5-propyl-2-thiouracil (PTU) also caused a slight interference at the concentration tested. This level would probably be higher than in a neonate on this drug.